APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1984, p. 601–606
0099-2240/84/040601-06$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 47, No. 4

Shake Flask Biodegradation of 14 Commercial Phthalate Esters

RICHARD H. SUGATT,[1] DEAN P. O'GRADY,[1] SUJIT BANERJEE,[1] PHILIP H. HOWARD,[1]* AND W. E. GLEDHILL[2]

Life and Environmental Sciences Division, Syracuse Research Corporation, Syracuse, New York 13210,[1] and Monsanto Company, St. Louis, Missouri 63166[2]

Received 29 September 1983/Accepted 4 January 1984

An acclimated shake flask CO_2 evolution test was used to study the biodegradability of 14 commercial phthalate esters that are commonly used as plasticizers. Both CO_2 evolution (ultimate biodegradation) and loss of parent phthalate esters (primary biodegradation) were measured. With only a few exceptions, primary biodegradation was 90% or higher, and ultimate biodegradation was in excess of 55% of theoretical results in 28 days. The results showed that all of the commercial phthalate esters were susceptible to biodegradation by mixed populations of microorganisms from natural sources. The results also provide considerable insight into the utility and reproducibility of a standard biodegradation test that is being recommended for widespread screening of chemicals.

Although the biodegradability of a limited number of commerical phthalate esters (PAEs) has been studied in natural waters (7, 8, 13, 14), activated sludge (4, 8, 10), hydrosoil (5), and defined aqueous media (8, 9), a broad range of commercial PAEs has never been studied in a single test system. To determine the relative biodegradability of these compounds, we tested 14 PAE composite samples representative of major commercial PAE products by a slightly modified CO_2 evolution procedure (3). This procedure has been recommended under the *Toxic Substances Control Act Premanufacturing Notice* testing guidelines (11). This investigation also was designed to provide an evaluation of the reproducibility of the CO_2 evolution procedure.

MATERIALS AND METHODS

General method. The CO_2 evolution procedure consisted of exposing the chemical to an acclimated inoculum in a defined medium over a 28-day period (3). The inoculum was prepared from soil, sewage microorganisms, and PAE in a 2-week acclimation period before the test initiation. The test chemicals were then added to replicate flasks containing the inoculated medium, and the flasks were incubated in the dark with shaking. Some of the replicate flasks were equipped with a suspended reservoir containing barium hydroxide solution, which was periodically sampled and titrated to measure the amount of CO_2 evolved. Differences in the CO_2 evolution between control flasks containing no test chemical and flasks containing PAEs were used to determine the extent and rate of ultimate biodegradation. At the beginning, middle, and end of the test, the entire contents of replicate flasks were extracted with hexane, and the concentrated extract was analyzed by gas chromatography with a flame ionization detector to determine the amount of PAE remaining (primary biodegradation).

The studies were conducted in four sets. Each set involved six PAEs, at least one blank control, and at least one glucose control. Sets were designed to include PAEs with high, medium, and low water solubility (1). For each PAE in a set, seven flasks were used. Three flasks were equipped for CO_2 evolution, and four were used for primary biodegradation measurements. Each set included di(2-ethylhexyl) phthalate (DEHP) to monitor interset reproducibility. The blank controls in set no. 3 leaked, and therefore the ultimate biodegradation for that set could not be measured; a fourth set was subsequently run. The acclimation medium for a set was prepared by following the acclimation procedure for each of the six PAEs in the set, pooling the acclimate for the set, and then adding the pooled acclimate to each test flask.

Chemicals. Commerical samples of 14 PAEs were supplied by U.S. manufacturers for testing. If more than one manufacturer produced the individual PAE, the PAE product provided was an equal proportion blend. Before testing, the PAEs were analyzed to confirm that they were within commercial specification limits. The PAEs tested were: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), butyl benzyl phthalate (BBP), dihexyl phthalate (DHP), butyl 2-ethylhexyl phthalate (BOP), di(*n*-hexyl, *n*-octyl, *n*-decyl) phthalate (610P), DEHP, diisooctyl phthalate (DIOP), diisononyl phthalate (DINP), di(heptyl, nonyl, undecyl) phthalate (711P), diisodecyl phthalate (DIDP), diundecyl phthalate (DUP), and ditridecyl phthalate (DTDP).

Acclimation procedure. The acclimation medium was prepared by adding 1.0 g of organically rich fresh soil, 2.0 ml of fresh aerated mixed liquor obtained from an activated sludge treatment plant, and 50 ml of raw domestic influent sewage with 1 liter of mineral salts medium prepared similarly to that used by Gledhill (3). The mineral salts medium was prepared by adding 1 ml each of solution 1 (NH_4Cl, 35 g/liter; KNO_3, 15 g/liter; $K_2HPO_4 \cdot 3H_2O$, 75 g/liter; $NaH_2PO_4 \cdot H_2O$, 25 g/liter), solution 2 (KCl, 10 g/liter; $MgSO_4$, 20 g/liter; $FeSO_4 \cdot 7H_2O$, 1 g/liter), and solution 3 ($CaCl_2$, 5 g/liter; $ZnCl_2$, 0.05 g/liter; $MnCl_2 \cdot 4H_2O$, 0.5 g/liter; $CuCl_2$, 0.05 g/liter; $CoCl_2$, 0.001 g/liter; H_3BO_3, 0.001 g/liter; MoO_3, 0.0004 g/liter) to 1 liter of aerated distilled water. This medium was mixed for 15 min and filtered through glass wool. The filtrate was supplemented with 25 mg each of vitamin-free Casamino Acids and yeast extract (Difco Laboratories, Detroit, Mich.) per liter. The organically rich soil was collected just upland from a freshwater marsh in Berry Park, Syracuse, N.Y. Soil was obtained from a depth of ca. 10 cm and screened through a seive with 2-mm openings. Mixed liquor and raw influent sewage were obtained from the Meadowbrook-Limestone Treatment Plant, Fayetteville, N.Y. This facility treats only domestic wastes. Soil and

* Corresponding author.

sewage samples were refrigerated at 4°C and used within 48 h of collection.

For each PAE, 1 liter of acclimation medium in a 2-liter Erlenmeyer flask was inoculated with a PAE concentration equivalent to 4 mg of carbon at the start of acclimation. The acclimation flasks were sealed and incubated in the dark on a Gyrotory shaker at 120 rpm and 22 ± 2°C. An additional test compound equivalent to 8 mg of carbon was added on day 7 and again on day 11 during the 14-day acclimation period. At the end of the acclimation period, the contents of all of the acclimation flasks in a set were pooled and filtered through glass wool to provide a common inoculum for the primary and ultimate biodegradation tests.

Primary and ultimate biodegradation procedures. On the day before beginning each set of tests, 900 ml of distilled water was added to each of the test flasks, which were then supplemented with 1 ml each of solutions 1, 2, and 3. Flasks were sealed and sparged overnight with 70% oxygen in nitrogen to remove dissolved CO_2. Four 2-liter Erlenmeyer flasks for specific PAE analysis and three 2-liter CO_2 evolution flasks (Ace Glass Co., Vineland, N.J.) were used for each PAE. Each set also utilized CO_2 evolution flasks for at least one glucose control and at least one blank control.

To begin the tests, a measured weight (nominal 20 mg) of test compound (PAE or glucose) on a preweighed glass slide was added to the appropriate flasks. Then, 100 ml of the pooled acclimation inoculum was added to the seven flasks for each PAE in a set and to the glucose and blank control flasks. The blank control flask received neither PAE nor glucose. After the addition of the test compound, the pH was measured and was 7.0 ± 0.2 without adjustment. The contents of two of the Erlenmeyer flasks for each PAE were immediately extracted for specific chemical analysis at time zero to determine the percent recovery. The remaining flasks were sparged with 70% oxygen in nitrogen (CO_2 free) for 5 min to remove CO_2 and sealed, and 10 ml of 0.2 N $Ba(OH)_2$ base was added to the central suspended reservoir of the CO_2 evolution flasks by injection through the septum seal. The flasks were incubated in the dark on a Gyrotory shaker at 120 rpm and 22 ± 2°C. Periodically during the test, base was removed by syringe from the suspended reservoirs for analysis. The appropriate sampling schedule was dictated by the rate of CO_2 evolution, which was qualitatively judged from the amount of $BaCO_3$ precipitate present in the absorber. After sampling, the flasks were sparged for 5 min, and fresh base was added. Specific PAE analysis was conducted on the contents of the remaining duplicate Erlenmeyer flasks for each PAE in a set at the time when the CO_2 evolution results indicated that at least 50% ultimate biodegradation may have occurred. On day 28, the medium was acidified to pH 3 with 20% H_2SO_4 to convert residual carbonates to CO_2. Base was removed for analysis on the next day, and the entire contents of the three CO_2 evolution flasks for each PAE in a set were extracted for specific chemical analysis.

Analytical procedures. To quantify evolved CO_2 trapped as $BaCO_3$, the entire contents of the suspended reservoirs were removed by syringe. The reservoirs were rinsed twice with two 10-ml portions of CO_2-free distilled water. These rinses were added to the sample, and the sample was titrated with standardized 0.05 N HCl to a phenolphthalein endpoint (pH 8.5 to 9.0).

To measure primary biodegradation by specific PAE analysis, the entire contents of each flask were added to a 1-liter separatory funnel. The flask was rinsed three times with 50-ml portions of hexane which were added to the funnel. The water was then extracted with the above 150 ml of hexane in the separatory funnel on an automatic shaker. This procedure was repeated twice. The pooled hexane extract was passed through anhydrous preextracted NaCl and Na_2SO_4 and added to a Kuderna Danish apparatus. The extract was allowed to evaporate until the extract volume was reduced to ca. 5 ml. These samples were then analyzed by gas chromatography with a Hewlett-Packard 5840 chromatograph equipped with a flame ionization detector, a column of 3% OV-1 on Supelcoport 100/120 mesh, and appropriate standards. Analytical studies were conducted to determine whether the PAEs could be detected at 1% of the initial added amounts. The 20 mg in each flask was concentrated after extraction to ca. 5 ml of hexane (final concentration, 4,000 mg/liter). Solutions (40 mg/liter; equivalent to 1% of the initial concentration in shake flask medium) were analyzed for all of the PAEs. This concentration was detectable for all of the PAEs except DTDP. DTDP exhibited a broad chromatographic peak and as a result was detectable only to ca. 2,000 mg/liter. This was equivalent to 50% of the initial concentration, which only allowed monitoring of primary biodegradation to 50% for DTDP.

Data analysis. Primary degradation results are expressed as the percentage of initial PAE which was lost during the test period. Means and standard deviations were calculated when possible (i.e., when <99% primary degradation had occurred). Ultimate biodegradation results are expressed as the percentage of theoretical CO_2 evolution in each flask. Means, standard deviations, and 95% confidence intervals were also calculated. The CO_2 evolution data from each flask were used to calculate an ultimate biodegradation rate constant by nonlinear regression analysis which fit the data to the model suggested by Larson (6). This analysis was conducted by a computer-based iterative procedure described previously (12). The individual rate constants calculated for each of the triplicate runs with each PAE in a set were used to calculate a mean rate constant and its standard deviation for that PAE. The difference between mean values was determined by a comparison of the 95% confidence intervals. Mean values were judged to be statistically different if their 95% confidence intervals did not overlap.

RESULTS AND DISCUSSION

The CO_2 evolution data, estimated rate constants and half-life, and percentages of primary degradation for the PAEs and glucose tested in sets 1 through 4 are presented in Table 1. It was originally intended to run all of the PAEs in three sets; however, both of the blank units in set 3 developed leaks to the atmosphere, which resulted in high levels of $BaCO_3$ precipitation. As a result, a fourth set was run in which CO_2 evolution data were collected, as in the previous sets, but in which specific PAE analysis was conducted only at the end of the test on the contents of the triplicate CO_2 evolution flasks. The inoculum used for BBP in set 4 was acclimated only to BBP to see whether this made a difference in the lag phase for this PAE. This was undertaken because BBP was the only monoaromatic alcohol phthalate used and may have had a long lag phase in set 2 due to the pooled inoculum being acclimated to mostly dialkyl phthalates.

The specific PAE analysis recoveries were generally 90 to 100%. DMP had a low value (44%) in set 1 which was inaccurate due to spillage during extraction. Identical procedures were used to check these recoveries with the inoculum and medium prepared during set 4, and these recoveries were 102 and 99%, respectively.

TABLE 1. Summary of biodegradation values for 14 PAEs[a]

Chemical	Set	Mol wt	Measured % carbon	% Primary biodegradation on day: 2–3	6–7	9	13–14	20–21	28	Lag phase (days)	Rate constant ($days^{-1}$)	Half-life (days)	% Primary biodegradation
DMP	1	194.2	62.2	6.0 (2)	69 (9)	77 (12)	81 (12)	85 (13)	86 (12)	2.7 (0.06)	0.364 (0.015)	1.90 (0.08)	>99
DEP	1	222.2	65.1	11 (2)	76 (1)	83 (2)	88 (3)	93 (3)	95 (2)	2.3 (0.1)	0.315 (0.036)	2.21 (0.26)	>99
DBP	1	278.3	68.9	5 (2)	15 (4)	17 (5)	23 (5)	40 (8)	57 (15)	4.5 (5.6)	0.050 (0.020)	15.4 (5.7)	89.8 (14.4)
BBP	2	312.4	73.6	2 (2)	4 (4)	4 (4)	6 (3)	18 (7)	43 (11)	15.6 (3.5)	0.043 (0.020)	19.4 (10.6)	77.7 (16.3)
	4			1 (2)	8 (1)	12 (1)	42 (9)	71 (13)	88 (7)	7.2 (1.1)	0.062 (0.038)	15.3 (10.9)	>97
Mean for BBP									43[b]		0.043[b]	19.4[b]	77.7[b]
BOP	4	334.5	73.4	18 (3)	53 (1)	67 (3)	79 (1)	84 (3)	87 (3)	1.0 (0.6)	0.153 (0.016)	4.55 (0.45)	>99
DHP	2	334.5	72.4	5 (1)	51 (5)	64 (5)	74 (6)	77 (5)	77 (5)	1.6 (0.2)	0.241 (0.041)	2.93 (0.55)	>99
610P	4		74.6	8 (0)	43 (2)	57 (4)	76 (3)	86 (4)	90 (4)	2.1 (0.2)	0.131 (0.010)	5.30 (0.43)	>99
DIOP	1	390.5	74.0	2 (1)	21 (8)	27 (10)	40 (15)	53 (19)	57 (21)	2.2 (1.1)	0.082 (0.021)	8.82 (2.43)	>99
DEHP	1	390.5	74.4	4 (3)	40 (5)	56 (8)	71 (13)	81 (15)	87 (13)	2.7 (0.3)	0.153 (0.020)	4.55 (0.58)	>99
	2			2 (1)	23 (2)	43 (3)	60 (3)	67 (4)	73 (3)	1.7 (0.3)	0.102 (0.002)	6.77 (0.19)	>99
	4			9 (1)	45 (2)	62 (3)	80 (1)	88 (0)	92 (3)	2.1 (0.06)	0.140 (0.008)	4.94 (0.27)	>99
Mean for DEHP									86		0.136	5.25	>99
DINP	2	418.6	75.0	2 (0)	5 (1)	12 (2)	41 (1)	57 (1)	62 (2)	7.1 (0.5)	0.131 (0.013)	5.31 (0.49)	>99
711P	4		70.2	9 (2)	48 (2)	64 (3)	83 (3)	94 (2)	98 (2)	2.1 (0.2)	0.137 (0.004)	5.03 (0.14)	>99
DIDP	2	446.7	76.1	1 (1)	5 (2)	17 (7)	39 (12)	53 (9)	56 (7)	4.9 (0.2)	0.088 (0.041)	9.6 (5.9)	>99
DUP	1	474.7	76.6	3 (1)	30 (2)	42 (5)	57 (4)	68 (3)	76 (2)	2.7 (0.2)	0.115 (0.024)	6.17 (1.17)	>99
DTDP	2	530.8	77.4	1 (0)	6 (6)	10 (10)	20 (12)	28 (12)	35 (13)	3.8 (2.8)	0.030 (0.017)	27.8 (13.3)	>50
	4			2 (2)	9 (1)	12 (1)	22 (3)	32 (7)	39 (8)	2.1 (1.2)	0.029 (0.014)	27.6 (10.7)	>50
Mean for DTDP									37		0.029	27.7	>50
Glucose	1,2,4	180.2	40.0[c]	34 (15)	72 (8)	80 (10)	84 (10)	91 (18)	92 (15)	0.35 (0.70)	0.227 (0.038)	3.11 (0.50)	

[a] Mean (standard deviation).
[b] Only from set 2 since set 4 BBP had a different acclimation procedure.
[c] Calculated.

In general, all of the PAEs were biodegraded rapidly as measured by loss of the parent molecule (primary biodegradation) and percentage of CO_2 evolution (ultimate biodegradation). The primary biodegradation at 28 days was >99%, except for DBP, BBP, and DTDP, which were 68 to >99%, 59 to 89%, and >50%, respectively. Similar conclusions could be reached from the ultimate biodegradation data. All of the PAEs tested with pooled acclimated inoculum had >50% theoretical CO_2 evolution at day 28 with the exception of BBP and DTDP, which were 43 and 37%, respectively. However, BBP and DTDP were still degrading at day 28. The 95% confidence limits indicate that many of the differences between the mean ultimate biodegradation and rate constant values are not statistically significant.

Variation in CO_2 evolution values among the triplicates or duplicates for each PAE and glucose in the sets can be examined by expressing the standard deviation as a percentage of the mean values obtained from the individual runs in a set. At day 28, the standard deviations of CO_2 evolution data ranged between ±2 and ±37% of the respective means. At day 28, the mean and median of the standard deviations were ±12 and ±5%, respectively. The ratio between the highest and lowest individual CO_2 evolution value among duplicates or triplicates on day 28 ranged between 1.0 and 2.1 and averaged 1.3. This indicates that individual values for a chemical varied by no more than about twofold. The rate constant data were more variable. The range, mean, and median of the standard deviations (expressed as a percentage of the mean) were 2 to 57, 21, and 14.5%, respectively. The ratio between the highest and lowest individual rate constant among duplicates or triplicates ranged between 1.0 and 2.9 and averaged 1.6, indicating that the individual rate constants for a chemical varied about twofold on the average. All of the individual CO_2 and rate constant values for a chemical in a set were within two standard deviations of the set mean calculated for that chemical, indicating that outlying data points were absent.

Information concerning interset variability of a chemical is available with CO_2 evolution data and rate constants for DEHP, DTDP, and glucose and with primary biodegradation data for the aforementioned chemicals and DBP, 610P, DIOP, and 711P. Mean values for CO_2 evolution within sets ranged between 73 and 92% for DEHP, 35 and 39% for DTDP, and 71 and 105% for glucose. Mean rate constants ranged between 0.102 and 0.153 for DEHP, 0.029 and 0.030 for DTDP, and 0.204 and 0.274 for glucose. Mean values for primary biodegradation at day 28 were >99% for both sets of 610P, DIOP, 711P, and four DEHP sets; >50% for both sets of DTDP; between 77.7 and 97.2% for BBP; and between 80.6 and >99% for DBP. The largest interset variation between mean values was the twofold difference in CO_2 evolution for the two BBP sets. None of the mean CO_2 evolution values for a chemical in a set were significantly different from the means for the same chemical in another set nor from the grand mean of the pooled data from all sets for that chemical. All individual values for a chemical were within two standard deviations of the set means and the pooled means for that chemical, indicating that outlying data points were absent even when experiments were repeated in separate sets.

The largest interset variation of CO_2 evolution occurred for the two BBP sets and is probably due to the different acclimation procedures. This illustrates the impact of acclimation procedures on two related parameters: lag period and CO_2 evolution in a fixed period of time. If the microbial seed is not acclimated (population of degraders grown up, enzymes induced, etc.) by the start of the test, there will be an increase in the lag period and a decrease in CO_2 evolution at day 28. In contrast, the rate constant for BBP does not appear to be affected very much by the different acclimation procedures, suggesting that rate constants are a better way of reporting and comparing biodegradation results, as has been suggested by Larson (6). In this study, the rate constant is further complicated by the fact that for many PAEs the amount added is above the water solubility.

As summarized in Table 2, primary biodegradation has been investigated previously in other test systems with seven of the PAEs tested in the present study (4, 5, 8, 9). The primary biodegradation results of this study agree well with those obtained previously with DMP, DEP, DBP, and BBP, although our results with DBP and BBP (90 and 77% mean primary biodegradation, respectively) are somewhat lower than those obtained previously (92 to 100%) in activated sludge, static cultures, and river water. The slightly lower values in our test system may be explained by the fact the DBP and BBP were still degrading at day 28. Our results with DEHP, 711P, and DUP (all having >99% primary biodegradation) are generally higher than those found previously. Primary biodegradation of DEHP was 10% in river water, 47% in hydrosoil, 74 to 91% in activated sludge, and 93 to 95% in static cultures. 711P and DUP degraded 0 and 10%, respectively, in river water and 51 and 37%, respectively, in activated sludge. Although previously published primary biodegradation information is not available for seven of the tested PAEs (BOP, DHP, 610P, DIOP, DINP, DIDP, and DTDP), the comparative results with the seven other PAEs suggest that the shake flask test system yields primary biodegradation values which are equal to or greater than those produced in the other test systems. Because primary biodegradation of all of the PAEs except DTDP reached 77% or higher, no relationship between molecular weight (or other characteristics) and primary biodegradation can be established.

As described above, the intraset and interset variability in CO_2 evolution values and rate constants was rather high. As a result, many of the mean values were not significantly different from some others. There appears to be a general trend for decreased percentages of CO_2 values and rate constants with the higher-molecular-weight PAEs. DTDP, for instance, had mean CO_2 evolution and rate constant values which were significantly lower than those of 9 and 10, respectively, of the lower-molecular-weight PAEs. Conversely, the mean rate constants for DMP and DEP were significantly higher than those of 9 and 11, respectively, of the higher-molecular-weight compounds. This trend is very weak, as can be seen in Table 2, which lists the PAEs and biodegradation values in order of increasing molecular weight.

Previous CO_2 evolution studies have been conducted with only three of the PAEs (BBP, DEHP, and 711P) used in this study (Table 2). Saeger and Tucker (8) reported that 27-day CO_2 evolution was 95% for BBP, 86% for DEHP, and 85% for 711P by the Sturm test. Our mean values were appreciably lower for BBP (43%), identical for DEHP (86%), and somewhat higher (98%) for 711P. Considering set 4 in which BBP received separate acclimation, our data are in excellent agreement with those of Saeger and Tucker (8). Nevertheless, both sets of values were 40% or greater, which is sufficient to establish that these compounds exhibit considerable ultimate biodegradability.

Rate constants for primary biodegradation in activated sludge and natural water have been reported for five of the

TABLE 2. Comparison of percentage of biodegradation results with previous studies

PAE	Code[a]	Activated sludge[b] at 48 h (% degraded)	Semicontinuous activated sludge[c] (% degraded)	River water[c] at wk 1 (% degraded)	Hydrosoil[d] at wk 2 (% degraded)	Static culture biodegradation[e] at day 7 (% degraded)	CO_2 evolution[c] at day 27 by Sturm test (% of theoretical CO_2)	CO_2 evolution[f] at day 28 with shake flask (% of theoretical CO_2)	Primary biodegradation[f] at day 28 with shake flask (% degraded)
Dimethyl	DMP					100		86	>99
Diethyl	DEP					100		95	>99
Di-*n*-butyl	DBP				92	100		57	90
Butyl benzyl	BBP	99	96	95		100	95	43	77
Butyl 2-ethylhexyl	BOP							87	>99
Butylglycolbutyl			99	100					
Dihexyl	DHP							77	>99
Di(*n*-hexyl, *n*-octyl, *n*-decyl)	610P							90	>99
Di-*n*-octyl						92–94			
Diisooctyl	DIOP							57	>99
Di(2-ethylhexyl)	DEHP	91	74	10	47	93–95	86	86	>99
Diisononyl	DINP							62	>99
Di(heptyl, nonyl, undecyl)	711P		51	0			85	98	>99
Diisodecyl	DIDP							56	>99
Diundecyl	DUP		37	10				76	>99
Ditridecyl	DTDP							37	>50

[a] Codes are listed for the PAEs tested in this study.
[b] Graham (4).
[c] Saeger and Tucker (8).
[d] Johnson and Lulves (5).
[e] Tabak et al. (9).
[f] Results determined in this study.

PAEs tested in this study. Comparison of our results with these rate constants is difficult because the units for the first-order rate constants in activated sludge were not reported by Urushigawa and Yonezawa (10), and the rate constants reported by Baughman et al. (2) and Wolfe et al. (13) were second order and expressed in different units. In addition, our rate constants were based on CO_2 evolution rather than primary biodegradation. However, these studies indicate a general trend for the primary biodegradation rate constants to decrease with increasing molecular weight or, as in the case of di-*n*-octyl phthalate and DEHP, with increased branching. Our rate constant results suggest a similar trend with the obvious exceptions of DBP and BBP, which had rate constants that were among the lowest determined for all 14 PAEs.

Rubin et al. (7) has studied the rates of DEHP mineralization (ultimate biodegradation) in natural lake waters at low test chemical concentrations. In 60 days, they detected no mineralization of DEHP added to an oligotrophic lake sample and immediate mineralization of DEHP added to a eutrophic lake sample. They attributed this lack of mineralization in the oligotrophic lake to a lack of presence or activity of microbial populations capable of degrading DEHP. The test system used in our study is designed to screen compounds allowing for acclimation and with relatively low microorganism populations. It was recently recommended in the *Chemical Fate Test Guidelines* by the U.S. Environmental Protection Agency (11) as a test that "provides evidence that the test substance will be biodegradable in natural aerobic freshwater environments." The results of Rubin et al. (7) for DEHP suggest that extrapolation of results of screening biodegradation tests such as the one presented here to natural water systems requires the consideration of a number of factors, some of which may not be recognized yet. For instance, very little is known about what effect environmental conditions have on the process of acclimation in natural waters. Nevertheless, it seems reasonable to conclude that the commercial PAEs in general will be biodegraded in many of the microenvironments (e.g., aerobic and eutrophic waters) in which they are likely to be released.

Conclusion. Based on ultimate (CO_2 evolution) or primary (loss of parent compound) criteria, all of the PAEs were capable of undergoing extensive biodegradation in the test system used. Similar results have been noted in previous literature. The degree to or rate at which PAEs are biodegraded varies for different compounds, with the low-molecular-weight compounds degrading slightly faster than the higher-molecular-weight compounds (Table 2). With the exception of DBP and DTDP, all dialkyl phthalates showed primary biodegradation of 90% or higher and ultimate biodegradation in excess of 55%. The half-life for ultimate biodegradation for all dialkyl phthalates was <28 days. BBP, which represents a special structural case, is also biodegraded but not as readily with pooled inocula (78% primary, 43% ultimate) as with an inoculum acclimated only to BBP (97% primary, 88% ultimate). Thus, it can be stated that all of the major commercial PAEs appear to be relatively biodegradable in the test system utilized.

ACKNOWLEDGMENTS

This project was supported by the Chemical Manufacturers Association under contract PE 13.0-SFB-SRC.

Valuable discussions with V. Saeger of Monsanto Co. are appreciated.

LITERATURE CITED

1. **Banerjee, S., A. E. Dombrowski, and P. H. Howard.** 1983. Final report—measurement of the water solubilities of phthalate esters—April 1983; butyl 2-ethylhexyl phthalate addendum—July 1983. Syracuse Research Corp. report (L1533-06) to Chemical

Manufacturers Association contract PE 11.0-WS-SRC. Syracuse Research Corp., Syracuse, N.Y.
2. **Baughman, G. L., D. F. Paris, and W. C. Steen.** 1980. Quantitative expression of biotransformation rate, p. 105–111. *In* A. W. Maki, K. L. Dickson, and J. Cairns, Jr. (ed.), *Biotransformation and fate of chemicals in the aquatic environment*. American Society for Microbiology, Washington, D.C.
3. **Gledhill, W.** 1975. Screening test of assessment of ultimate biodegradability: linear alkylbenzene sulfonates. Appl. Microbiol. **30**:922–929.
4. **Graham, P. R.** 1973. Phthalate ester plasticizers—why and how they are used. Environ. Health Perspect. **3**:3–12.
5. **Johnson, B. T., and W. Lulves.** 1975. Biodegradation of di-*n*-butyl and di-2-ethyl hexyl phthalate in freshwater hydrosoil. J. Fish. Res. Board Can. **32**:333–339.
6. **Larson, R. J.** 1979. Estimation of biodegradation potential of xenobiotic organic chemicals. Appl. Environ. Microbiol. **38**:1153–1161.
7. **Rubin, H. E., R. V. Subba-Rao, and M. Alexander.** 1982. Rates of mineralization of trace concentrations of aromatic compounds in lake water and sewage samples. Appl. Environ. Microbiol. **43**:1133–1138.
8. **Saeger, V. W., and E. S. Tucker.** 1976. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microbiol. **31**:29–34.
9. **Tabak, H. H., S. A. Quave, C. I. Mashni, and E. F. Barth.** 1981. Biodegradability studies with organic priority pollutant compounds. J. Water Pollut. Control Fed. **53**:1503–1518.
10. **Urushigawa, Y., and Y. Yonezawa.** 1979. Chemico-biological interactions in biologial purification systems. VI. Relation between biodegradation rate constants of di-*n*-alkyl phthalate esters and their retention times in reverse phase partition chromatography. Chemosphere **5**:317–320.
11. **U.S. Environmental Protection Agency.** 1982. Chemical fate test guidelines. EPA 560/6-82-003. U.S. Environmental Protection Agency, Washington, D.C.
12. **Werstiuk, N. H., and S. Banerjee.** 1981. A simple procedure for the analysis of multisite hydrogen-deuterium exchange rates obtained by mass spectrometry. J. Org. Chem. **46**:471–473.
13. **Wolfe, N. L., L. A. Burns, and W. E. Steen.** 1980. Use of linear free energy relationships and an evaluative model to assess the fate and transport of phthalate esters in the aquatic environment. Chemosphere **9**:393–402.
14. **Wolfe, N. L., D. F. Paris, W. C. Steen, and G. L. Baughman.** 1980. Correlation of microbial degradation rates with chemical structure. Environ. Sci. Technol. **14**:1143–1144.